Analyzing Oysters Response to Environmental Stressors Using a Resazurin Assay

Colby Elvrum[1], Steven Roberts[2], Ariana Huffmyer[2]
2024 LSAMP Scientist Program
1. University of Washington, Chemical Engineering 2. University of Washington School of Aquatic and Fishery Sciences

LOUIS STOKES ALLIANCE FOR MINORITY PARTICIPATION
UNIVERSITY of WASHINGTON
Office of Minority Affairs & Diversity

Introduction

Oysters are an important part of the ecosystem and the ability to assess the effects of stress on them lets us better understand the influence of environmental change. Resazurin is a dye that is commonly used in cell viability, and it works by changing color as an organism consumes oxygen. In this assay, I used this attribute, and applied it to oysters to determine the effectiveness of a resazurin assay in evaluating the effects of environmental stressors on oysters.

Research questions:

1. Can resazurin be used to measure metabolism in oysters?
2. Does stress hardening change metabolic response in oysters?

Methods

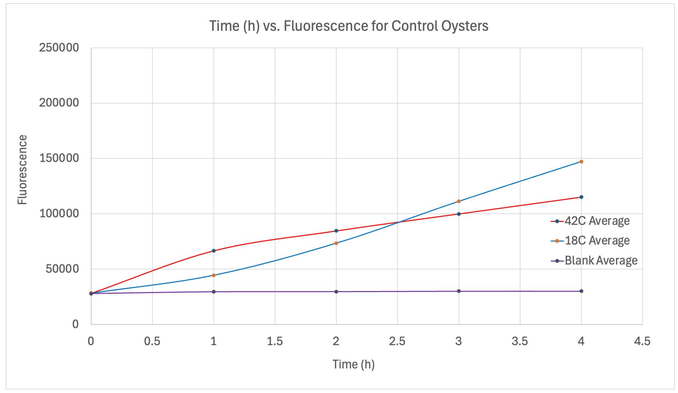
Several parameters were optimized by conducting multiple experiments and by changing one variable at a time. Parameters that were optimized include concentration of resazurin, volume, temperature, and form of stress.

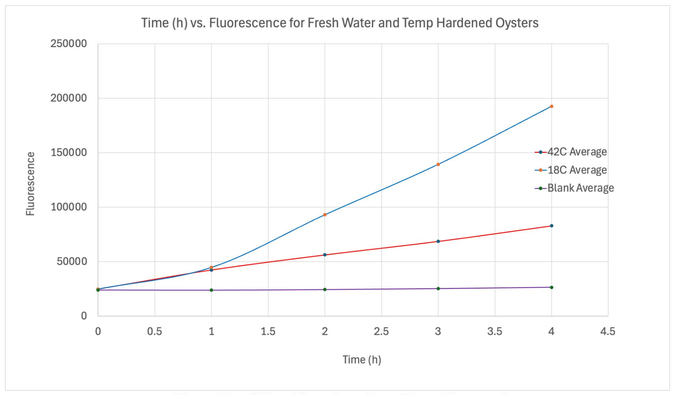
A structured experiment was then carried out where oysters with prior stress history were subjected to high temperature (42° C) or control (18°C) conditions in resazurin and fluorescence was measured every 1 h for 4 hours. Oyster survival was monitored for 24 hours following resazurin assays.

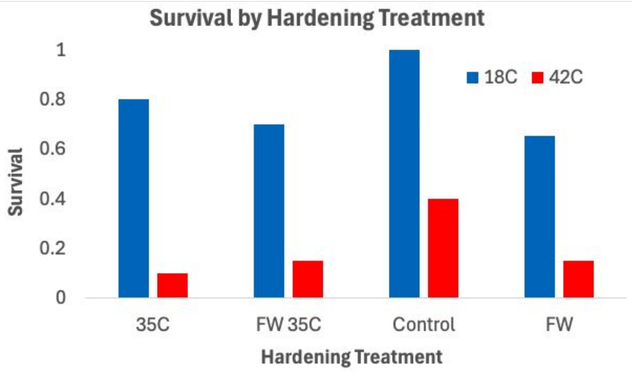
Results

Table 1. Optimal resazurin assay protocols

Protocol	Optimal Condition
Time of exposure	4 h exposure with measurements at 1 h intervals
Resazurin concentration	There was no noticeable difference in results with different dilutions
Sample preparation	Addition of antibiotic and antifungal to control microbial influence
Volume	1:4 oyster volume to resazurin volume ratio







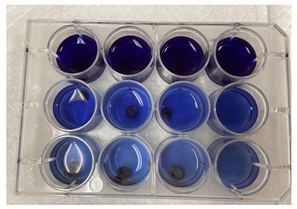


Image of Resazurin Dilution Protocol

Figure 1. Metabolic activity of the 42C oysters was higher in the control oysters than previously hardened oysters, and the opposite was true for the 18C oysters.

Figure 2. Prior exposure to hardening stress decreased oyster survival under 42°C.

Conclusions

Results show that resazurin is an effective indicator to evaluate environmental stressors, as there is initially a higher and more rapid increase in resazurin fluorescence when the oysters are exposed to elevated temperatures. This suggests that they have a higher metabolic rate initially, which then levels off as they begin to die. Additionally, oysters with high metabolic rates were more likely to show mortality.

Oysters previously exposed to hardening stress treatments showed a decrease in survival at 42°C which suggests they may become more sensitive to temperature changes

Implications

Some potential future steps could be to investigate how to scale up parts of the experiment, as everything has to be run manually. This could allow us to better evaluate the effects of previous hardenings, and could play a vital role in helping us further understand the effect of environmental changes on oysters due to changes in the environment like global warming.

Acknowledgements

This research was made possible by the National Science Foundation Grant Number 0901996.